FATE OF MALATHION IN AN ACTIVATED SLUDGE MUNICIPAL WASTEWATER TREATMENT SYSTEM

THESIS

Edward B. Walters, Major, USAF

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THESIS

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Abstract

Organophosphates (OPs) have been a primary component in pesticides the 1940's. OP human toxicity was discovered prior to World War II (WWII), and some were developed as Chemical Warfare Agents (CWAs) that remain a threat to national security today. Although originally designed for military applications, these compounds have been used successfully against civilian populations in the past. One of the most toxic of the OP CWAs is *O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate, commonly known as VX.

VX can be deployed in numerous ways; however, air dispersal is considered one of the most likely routes, in terms of inflicting damage on a population. Exhibiting high toxicity, high viscosity, low volatility, and relative resistance to degradation, VX is particularly persistent in the environment, making it especially dangerous. The military has decontamination procedures in the event of a chemical warfare incident. Standard operating procedures require contaminated personnel and property be decontaminated with a water solution containing bleach or soap. The resulting decontamination waste water should then be largely contained; however, it would eventually have to be disposed of either as hazardous waste or possibly treated at a wastewater treatment plant (WWTP).

There have been few studies to document the fate of these CWAs when subjected to a municipal wastewater treatment process. This research examined the fate of malathion, a surrogate of VX, in bench-scale sequencing batch reactors that simulate secondary treatment in a municipal activated sludge (AS) WWTP. Results show that

malathion may degrade in an AS WWTP as approximately 90% of an initial concentration of 4.25 mg L^{-1}.

Acknowledgments

Anything I have accomplished I owe to the guidance and support of many others. I wish to express my sincere gratitude to Major LeeAnn Racz for her unwavering support, encouragement, and exceptional guidance. As an advisor and professor, you are tops, and I will always be indebted to you. To my colleague Lieutenant Allen Janezcko, I could not have asked for a better teammate or friend. We accomplished much more together than I could have conceived alone. Two heads are definitely better than one. To Captains Justin DeLorit and Steve Schuldt, I owe my success to the solid foundation you provided. You were a vital source of information and I consider myself fortunate to have worked with both of you. I would, also, like to thank my sponsor Matthew Magnuson, from the U.S. Environmental Protection Agency for the support provided to me in this endeavor. Last but not least, I wish to thank my wife for providing me with the love and support needed in order to continually push myself to succeed. I am especially grateful for you giving birth to our daughter and shouldering the parenting load during my coursework. You made everything possible. This experience has made me a better friend, teammate, and professional.

Edward B. Walters

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FATE OF MALATHION IN AN ACTIVATED SLUDGE MUNICIPAL WASTEWATER TREATMENT SYSTEM

I. Introduction

Background

Organophosphates (OPs) inhibit acetylcholinesterase, an enzyme essential to nerve function in insects, humans, and many other animals. Acetylcholinesterase is an enzyme present in nervous tissue that metabolizes acetylcholine, a neurotransmitter, preventing excessive firing at neuromuscular junctions. Once popular components in flame retardants, plasticizers, emulsifiers, and lubricating oil additives, OPs are currently predominantly used in insecticides, and pesticides (Szinicz, 2005). The German Ministry of War began exploring the potential for OPs as chemical warfare agents upon discovery of their high mammalian toxicity in the 1930's (Szinicz, 2005). That research led to the development of nerve agents such as tabun, sarin, soman, and ultimately many years after WWII, VX(*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate). Nerve agents are OP chemical warfare agent (CWA) compounds created by condensing an acid such as phosphoric or phosphonic acid with an alcohol (Szinicz, 2005).

The toxicity of OPs affects and may disable the normal operation of the nervous system. Acetylcholine (ACh) is a common neurotransmitter found in the central and peripheral nervous systems (Cannard, 2006). Its function is to control muscular contraction. In a system functioning normally, ACh is released from the axon terminal and binds with the receptor on the post-synaptic membrane causing muscular contraction (Cannard, 2006). The enzyme acetylcholinesterase (AChE) terminates the action by

cleaving the ACh into choline and acetic acid (Cannard, 2006). In a system with nerve agent poisoning, the nerve agent inhibits the AChE from terminating the action of the ACh by irreversibly binding with the AChE (Cannard, 2006). The ACh continues to release and accumulate in the synapse (Cannard, 2006). As a result, the muscles continue to contract, which causes nerve agent symptoms (Cannard, 2006).

Signs and symptoms of acute nerve agent poisoning vary from minor to severe depending on the dose received and duration of exposure. Common minor manifestations include: dizziness, lethargy, miosis, vomiting, abdominal cramps, diarrhea, muscle fasciculation or weakness, and increased salivation, lacrimation, urination, and perspiration (Casarett, Doull, & Klaassen, 2007). Severe manifestations include: mental confusion, incontinence, bronchial secretion, bradycardia or tachycardia, convulsions, coma, hypotension or transient hypertension, and paralysis (Casarett, Doull, & Klaassen, 2007). Death occurs most often due to respiratory failure or bronchoconstriction (Gallo & Lawryk, 1991; Lotti, 2000, 2001).

Despite their development for military purposes, nerve agents have been used against civilian populations in the past. Nerve agents were not used on enemy forces during WWII despite the mass development and production of the CWAs from the 1930s to 1950s (Szinicz, 2005). The only casualties of nerve agent poisoning recorded during WWII were inmates of concentration camps and plant workers where the agents were produced (Wills & DeArmon, 1954). The Germans tested the nerve agents on inmates to investigate their intoxicating effects and develop antidotes (Wills & DeArmon, 1954). Between 1983 and 1988, Iraqi military forces deployed tabun and sarin against Iran (Smart, 1997; Newmark, 2004). Iraq deployed nerve agent again between 1987 and 1988

in support of their campaign against the Kurds (Szinicz, 2005). Japan suffered sarin attacks organized by terrorist group Aum Shinrikyo in 1994 and 1995 in Matsumoto and Tokyo, respectively (Tu, 1996). The attacks by Aum Shinrikyo killed or injured approximately 2000 civilians (Wiener & Hoffman, 2004; Bowler and others, 2001; Sidell, 1997). Aum Shinrikyo attacked again in 1995, deploying VX on three separate occasions targeting judges (cns.miis.edu, 1996; Smithson & Levy, 2000). Seven civilians were killed while the judges and 141 other civilians sustained nonfatal injuries (cns.miis.edu, 1996; Sidell, 1997; Smithson & Levy, 2000). The United States began decommissioning its nerve agent stockpiles in the 1990's (Wiener & Hoffman, 2004). Since then, there have been periodic reports of unintentional exposures generally due to mishandling (Wiener & Hoffman, 2004). The most recent known nerve agent exposure occurred in 2004 during Operation Iraqi Freedom when two US Army Soldiers came into contact with an Iran-Iraq war sarin shell and experienced nonfatal symptoms (McDonough et al., 2008).

Sarin has been deployed the most throughout history. However, VX is widely considered to be one of the most lethal nerve agent (Szinicz, 2005). VX has a LD_{50} [dosage (milligrams toxicant per 70 kilogram person) causing death in 50% of an exposed population] much lower than other nerve agents. The LD_{50} of VX and the next lowest nerve agent, soman, is 10 mg/70 kg and 350 mg/70kg by dermal absorption, respectively (Sidell, 1997). It also remains intact on surfaces, where as other nerve agents will evaporate unless shielded from the environment (Robinson, 1971; Wills & DeArmon, 1954). VX has a hydrolysis half-life of 1,000 hours at pH 7, and an extremely low volatility of 10.5 mg/m^3 (Sidell, 1997; Munro et al., 1999). In comparison, soman

has the next lowest hydrolysis half-life of 60 hrs at pH 6, respectively (Munro et al., 1999). Tabun has the second lowest volatility of all nerve agents at 610 mg/m^3 (Munro et al., 1999). Sarin has the highest LD_{50} and volatility at 1,700 mg/70kg and 22,000 mg/m^3, respectively (Munro et al., 1999). VX is, by large margin, the most toxic, least volatile, and most persistent nerve agent in the environment..

Standard operating procedures for responding to CWA incidents dictate that contaminated personnel, property, and surfaces would be decontaminated with a water solution possibly containing bleach (Eng et al., 2002). The resulting decontamination waste water should then be largely contained to avoid further spread of contamination. However, inability to completely contain the decontamination waste water is inevitable. If the attack was airborne, buildings, surface water sources, and a large, diverse land area may be contaminated. Some contamination may find its way into the sanitary sewer. Ultimately, the decontamination wastewater must be disposed of as hazardous waste or possibly treated at a WWTP. There are no studies that document the fate of VX or its degradation products in a municipal WWTP , although a few relevant studies related to degradation of OP compounds in WWTP are discussed below. Note that biological degradation has been investigated for chemical demilitarization operations, but these results can not necessarily be applied in a straightforward manner to WWTP since the biological degradation in chemical demilitarization operations can be highly engineered to be specific for degradation of OPs. OP concentrations would be lower in a CWA incident due to dilution and the treatment system, not specifically designed to degrade OP, will vary by municipality.

Municipal WWTPs generally consist of three treatment phases (Fig 1). The primary treatment phase is responsible for removing larger debris and sediment with grit chambers and bar screens. The secondary phase is responsible for the removal of organic carbon, nutrients, and ammonia (Droste, 1997). It consists of an aerobic reactor containing activated sludge (AS), which is flocculated biological growth and wastewater. The bulk of the treatment occurs in this phase. During the operation of a WWTP, a portion of the AS is wasted every 15 – 20 days approximately in order to maintain a healthy and efficient nitrifying community (Metcalf & Eddy, 2002). The growth rate of autotrophic nitrifiers is dependent on favorable environmental variables and is much slower than heterotrophic bacteria (Droste, 1997). The waste sludge may be used for farming or sent to a landfill (Droste, 1997). Wasting is an effective method of contaminant removal for hydrophobic compounds (Bondarenko & Gan, 2004; Thomas et al., 2009).

AS consists of heterotrophic and autotrophic bacteria. The heterotrophic bacteria obtain energy from the oxidation of the reduced organic matter (Droste, 1997). The primary products of oxidation of the organic matter are carbon dioxide (CO_2), water (H_2O), and ammonia (NH_3) (Droste, 1997). The autotrophic bacteria obtain energy from the oxidation of inorganic compounds (Droste, 1997). The autotrophic bacteria use the CO_2 as a carbon source for cell synthesis and oxidize NH_3 to nitrate (NO_3) in the process of nitrification (Droste, 1997). NH_3 is oxidized to an intermediate, hydroxylamine (NH_2OH), via the ammonia monooxygenase (AMO) enzyme during the nitrification process (Racz & Goel, 2010). The AMO causes the hydroxylation of alkenes producing primary and secondary alcohols (Hyman & Wood, 1983; Hyman, et al., 1988). It was

determined that organic compounds, such as estrogen and ethyl methylphosphonic acid, could be degraded in nitrifying AS through the cometabolism of AMO (Ren et al., 2007a; Shi et al., 2004; Vader et al., 2000; Schuldt, 2012).

The tertiary or final phase is responsible for removing the contaminants that are able to pass through the primary and secondary phases. This phase generally consists of filtration to decrease the total suspended solids in the effluent, chemical disinfection, and/or chemical nutrient removal. The effluent is then discharged to a surface water body. Compounds remaining post treatment will be discharged with the effluent or wasted with the sludge, which will introduce the untreated contaminants to the natural environment. In some cases, the surface water body receiving the treated effluent may be used as a drinking water source downstream.

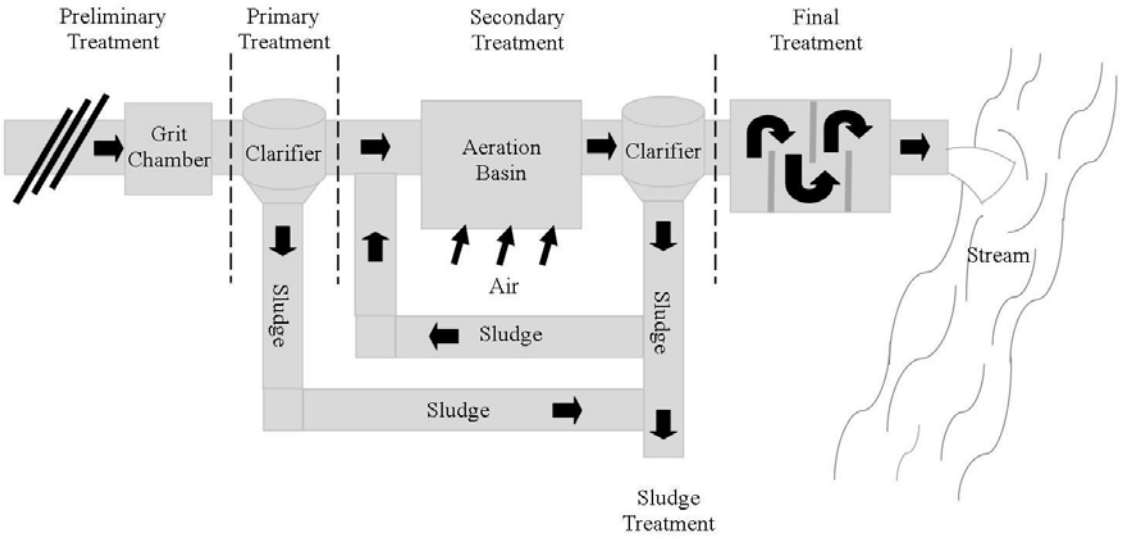

Figure 1: Wastewater Treatment Process

Problem Statement

In the event of a CWA release, it is possible that some CWA contamination may enter the influent of the local municipal WWTP. There have been few studies to

document the fate of VX or similar organophosphates when subjected to a municipal wastewater treatment process. Given the stability and solubility of VX, there is a potential for the contaminant to leave the plant untreated or partially treated. Receiving bodies of water may be contaminated as a result, affecting drinking water quality and possibly human health. Similarly, VX contaminated waste sludge applied as fertilizer may affect the quality of the resulting agricultural commodities.

Experimental Safety Constraints and Approach

VX is the one of the most lethal and persistent nerve agent. It is highly regulated in the United States and beyond the capability of our research laboratory to perform experiments with it. Accordingly, malathion was used as a surrogate in this research as some of its chemical and physical properties are similar to VX (Table 1).

Table 1: Identity, Chemical and Physical Properties of VX and Malathion (Munro et al., 1999; Sidell, 1997; Bartelt-Hunt et al., 2008; Newhart, 2006)

	Chemical	
Property/Parameter	**VX**	**Malathion**
Chemical Formula	$C_{11}H_{26}NO_2PS$	$C_{10}H_{19}O_6PS_2$
Physical State	Oily liquid	Liquid
Color	Light amber/amber	clear-amber
Melting point	-39°C	2.8 °C
Boiling Point	298°C	156 °C
Density, liquid (g/mL)	1.008 at 20°C	1.23 at 20°C
Vapor pressure (mmHg 20 or 25°C)	0.0007	3.38×10^{-6}
Volatility (mg/m^3)	10.5	*
Vapor Density (air=1)	9.2	11.4
Water solubility (g/L)	30	130
Hydrolysis rate (half-life at pH 7)	1,000 hr	100 hrs
Henry's constant (H, atm x m^3/mol)	3.5×10^{-9}	4.9×10^{-9}
Log Kow	2.09	2.36
Log Koc	2.5	2.77
LD50 (mg/70kg)	10	246 to 471

* - not available

Malathion, introduced in 1950 by American Cyanamid Co., is a nonsystemic OP insecticide (Martin & Worthing, 1977). Its low acute mammalian toxicity is due to its selective hydrolytic degradation via mammalian carboxylesterases, versus its oxidative activation to the potent acetylcholinesterase inhibitor malaoxon in insects (Brown et al., 1993). Over 16.7 million pounds of malathion is used annually in public health, residential, and agricultural settings in the United States (Newhart, 2006). An ideal VX simulant would mimic the relevant chemical and physical properties of the agent without the human toxicity (Bartelt-Hunt et al., 2008). Malathion has a much lower human toxicity with a minimum estimated LD_{50} of 246 mg/kg compared to 10 mg/kg for VX (Farago, 1967; Jusic & Milic, 1978). Sorption to and desorption from organic carbon is governed primarily by log K_{ow} value (Bartelt-Hunt et al., 2008). VX and malathion have similar log K_{ow} values at 2.09 and 2.36 respectively (Bartelt-Hunt et al., 2008). Chemical volatilization is governed by the Henry's constant. The Henry's constants for VX and Malathion have 10^{-9} magnitudes; therefore, both should partition similarly between water and air (Bartelt-Hunt et al., 2008; Munro et al., 1999). Biodegradability is governed primarily by chemical structure, and malathion is one of the most structurally similar simulants available (Bartelt-Hunt et al., 2008). Hydrolysis is primarily dependent on the presence of similar bonds at which the hydrolysis reaction occurs (Bartelt-Hunt et al., 2008). Under alkaline conditions, the hydrolysis of malathion proceeds at a rate reasonable for it to be used as a surrogate for VX (Bartelt-Hunt et al., 2008).

Research Questions

The objective of this research was to determine experimentally the capacity of municipal WWTP AS to degrade malathion, a surrogate for VX nerve agent, in bench-scale studies. Additionally, this study aimed to determine the mechanisms for removal of malathion, sorption capacity of the AS, and the effect of malathion degradation on both COD oxidation and nitrification.

The primary goals of this study were to determine:

1. The degradation of malathion by municipal WWTP AS
    a) The capacity for AS to degrade malathion
    b) Degradation kinetics of AS with respect to malathion
2. The capacity of the AS to sorb malathion.
3. The role of nitrifying and heterotrophic bacteria in the degradation of malathion.

Scope and Approach

This research sought to simulate the secondary treatment phase of municipal WWTP in the laboratory by designing and operating a 2.0 L sequencing batch reactor. This sequencing batch reactor, seeded with activated sludge from the Fairborn Water Reclamation Facility (FWRF), Fairborn, Ohio, was fed simulated wastewater and provided the AS samples used in the batch test experiments.

Batch test experiments were completed to determine sorption characteristics of malathion to activated sludge and the ability of AS to degrade malathion. The results

provided insight into the fate of VX in a municipal WWTP and the subsequent risk that may exist if the agent or products of its degradation exit the treatment process unchanged.

Significance

In the event of a CWA incident, contamination will be widespread. It is possible that VX decontamination wastewater may be sent, inadvertently or purposely, to a WWTP. There is a risk these compounds may leave a WWTP untreated or partially treated in the effluent or waste sludge and compromise the health of the surrounding natural water bodies. This poses significant human health concerns to communities downstream that may use connected water bodies as a source of food or water. It is important to understand the behavior of these OPs in AS biological systems in order to prevent the spread of contamination and reduce the risk human exposure in the event of a CWA release.

Preview

This thesis is written in the scholarly article format. Chapter 2 is a journal article produced from this research for future journal submission. Written as an independent chapter, it includes the following: abstract, introduction, materials and methods, results and discussion, and conclusions. Chapter 3 serves as a final discussion of the article conclusions. It summarizes the findings, research limitations, and presents opportunities for future research not discussed in Chapter 2.

II. Scholarly Article

Abstract

Organophosphate compounds are used as pesticides and in chemical warfare agents such as nerve agents. VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate) is one of the most toxic and environmentally persistent of these nerve agents. This research examined the fate of malathion, a pesticide and surrogate of VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate), in bench-scale activated sludge (AS) sequencing batch reactors. Sorption kinetics and sorption equilibrium isotherm experiments indicate that sorption to AS biomass was not a statistically important removal mechanism. However, approximately 90% of the initial 4.25 mg L^{-1} malathion concentration degraded primarily via heterotrophic activity.

Keywords: Organophosphate chemical warfare agents, malathion, activated sludge, VX biodegradation

Introduction

Organophosphates (OPs) have been a primary component in pesticides since the 1940's. Their human toxicity was discovered prior to World War II (WWII) and was developed as chemical warfare agents (CWAs), specifically nerve agents, which remain a threat to national security today. Although originally designed for military applications, these compounds have been used successfully against civilian populations in the past. The German Ministry of War began exploring the potential for OPs as CWAs upon discovery of their high mammalian toxicity in the 1930's (Szinicz, 2005). That research

led to the development of nerve agents such as tabun, sarin, soman and ultimately, VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate). OPs used as CWAs affect the normal operation of the nervous system by irreversibly binding to acetylcholinesterase (AChE) causing acetylcholine (ACh) to accumulate and continuously contract muscles (Cannard, 2006). VX is widely considered to be one of the most lethal nerve agent and is the focus of this research (Szinicz, 2005). VX has the lowest LD_{50} and the highest environmental persistence among nerve agents (Robinson, 1971; Wills et al., 1954).

Standard operating procedures for responding to CWA incidents dictate that contaminated personnel, property, and surfaces would be decontaminated with water (Eng et al., 2002). The decontamination waste water should then be largely contained to avoid further spread of contamination; however, some loss of containment is inevitable. Ultimately, the decontamination wastewater must be disposed of as hazardous waste or possibly treated at a wastewater treatment plant (WWTP). There are few studies that document the fate of VX or its degradation products in a municipal WWTP. However, it is expected that VX could be degraded by the wastewater treatment activated sludge (AS), removed from the waste sludge through sorption, or may pass through treatment unchanged posing risks to downstream populations.

VX is regulated by the Chemical Warfare Convention and was not able to be used for this study. Compared to other nerve agents, VX has one of the lowest LD_{50} and exhibits the highest persistence. (Robinson, 1971; Wills & DeArmon, 1954). Therefore, malathion was used as a surrogate for this research to mimic the relevant chemical and physical properties of the agent without the human toxicity (Bartelt-Hunt et al., 2008).

Malathion has a much lower human toxicity with a minimum estimated oral LD_{50} dosage of 246 mg/kg (milligrams toxicant per 70 kilogram person) compared to 10 mg/kg for VX (Farago, 1967; Jusic & Milic, 1978). VX and malathion have similar log K_{ow} values at 2.09 and 2.36 respectively (Bartelt-Hunt et al., 2008). The Henry's constants for VX and Malathion have 10^{-9} magnitudes; therefore, both should partition similarly between water and air (Bartelt-Hunt et al., 2008; Munro et al., 1999). Biodegradability is governed primarily by chemical structure, and malathion is one of the simulants most similar to VX (Bartelt-Hunt et al., 2008). Under alkaline conditions, the hydrolysis of malathion proceeds at a rate reasonable for it to be used as a surrogate for VX (Bartelt-Hunt et al., 2008).

This goal of this research was to investigate the capacity of municipal WWTP AS to degrade malathion in bench-scale studies. Additionally, we investigated the mechanisms for removal of malathion, namely sorption capacity of the AS, and the effect of malathion degradation on COD oxidation and nitrification.

Materials and Methods

Chemicals

Malathion (95% purity, CAS 121-75-5), product M00825, was purchased from Pfalz & Bauer (Waterbury, CT). The malathion was stored in a freezer at approximately -20°C. All other chemicals used in the experiments were high-pressure liquid chromatography or analytical grade. Cleaned, amber colored, autoclaved glassware was utilized during the experiments to prevent contamination and potential photolytic or biologic degradation.

Sequencing Batch Reactor Operation

The reactor was operated using a method adapted from Racz et al. (2010) and Schuldt et al. (2012), and is diagrammed in Figure 2. A 2.0L sequencing batch reactor (SBR) was constructed and seeded with AS from the Fairborn Water Reclamation Facility (FWRF), Fairborn, Ohio. The FWRF has a design flow of 6 million gallons per day (MDG) with an average daily rate of 4.4 MGD. The solids retention time (SRT) at the facility is 12.5 days in the summer and 11 days in the winter. FWRF averaged 97% and 99% for biological oxygen demand (BOD) and NH_3 removal, respectively.

Deionized water was utilized exclusively in the reactor and feed solutions to avoid interference from ions such as copper, calcium, and magnesium, and lead commonly found in tap water. The reactor was given two feeds (feed A and B) to simulate the composition of wastewater. The feeds provided the carbon, nitrogen, and nutrients necessary to achieve simultaneous chemical oxygen demand (COD) removal and nitrification. Feed A contained 44.6 g sodium bicarbonate ($NaHCO_3$) per liter of deionized water. The sodium bicarbonate ensured sufficient alkalinity existed in the system to support nitrification. Feed B, the wastewater simulant, contained the following ingredients per liter of deionized water: 3 g peptone, 1.25 g sodium acetate (NaOAc), 2.26 g ammonium chloride (NH_4Cl), 6.86 g magnesium chloride ($MgCl_2 \cdot 6H_2O$), 1.72 g calcium chloride ($CaCl_2 \cdot 2H_2O$), 0.6675 g potassium dihydrogen phosphate (KH_2PO_4) and 20mL of a trace element solution. Peptone is an easily metabolizable source of organic carbon with amino acids which provides nitrogen and energy for heterotrophic bacteria (Racz et al., 2010). Sodium acetate was added to the feed to simulate the

volatile fatty acids commonly found in municipal wastewater (Kindaichi et al., 2004). The trace elements in the mixture normally exist in municipal wastewater in minute quantities and are essential to the growth of the organisms in the AS. The composition of the trace element solution was adapted from Hesselmann et al. (1999) and contained the following per liter of deionized water: 5.46 g citric acid ($C_6H_8O_7$), 4.0 g hippuric acid ($C_9H_9NO_3$), 0.72 g nitriloacetic acid ($Na_3NTA \cdot 2H_2O$), 0.3 g sodium ethylene diamine tetra acetic acid ($Na_3EDTA \cdot 4H_2O$), 3.0 g ferric chloride ($FeCl_3 \cdot 6H_2O$), 0.5 g boric acid (H_3BO_3), 0.3 g zinc sulfate ($ZnSO_4 \cdot 7H_2O$), 0.24 g manganese chloride ($MnCl_2 \cdot 4H_2O$), 0.14 g copper sulfate ($CuSO_4 \cdot 5H_2O$), 0.06 g potassium iodide (KI), 0.06 g sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$), 0.06 g cobalt chloride ($CoCl_2 \cdot 6H_2O$), 0.06 g nickel chloride ($NiCl_2 \cdot 6H_2O$), and 0.06 g sodium tungstate ($Na_2WO_4 \cdot 2H_2O$).

The reactor was continuously operated in identical 12 hour cycles. With 1.33 L of AS in the reactor, the cycle began with the addition of 624 mL of deionized water, 38 mL feed A, and 8 mL feed B through a peristaltic pump increasing the total volume of each reactor to 2.0 L. Eleven and one-half hours of aeration followed the filling sequence. During this period, the mixed liquor was aerated with compressed air to ensure adequate mixing, AS contact time, and dissolved oxygen concentrations. The aeration and mixing stopped after the 11.5 hour aeration phase. The AS was allowed to settle for 20 minutes. The cycle ended by decanting 670 mL of the supernatant. The new cycle began with the addition of 624 mL of deionized water, 38 mL feed A, and 8 mL feed B. The resulting hydraulic retention time was 36 hours. The SRT was held at 20 days by wasting 100 mL of the AS from the SBR daily. The reactors averaged 91% COD removal and 99% NH_3-

N oxidation. The average total suspended solids (TSS) and volatile suspended solids (VSS) concentrations in the reactors were 1562 mgL^{-1} and 1306 mgL^{-1}, respectively.

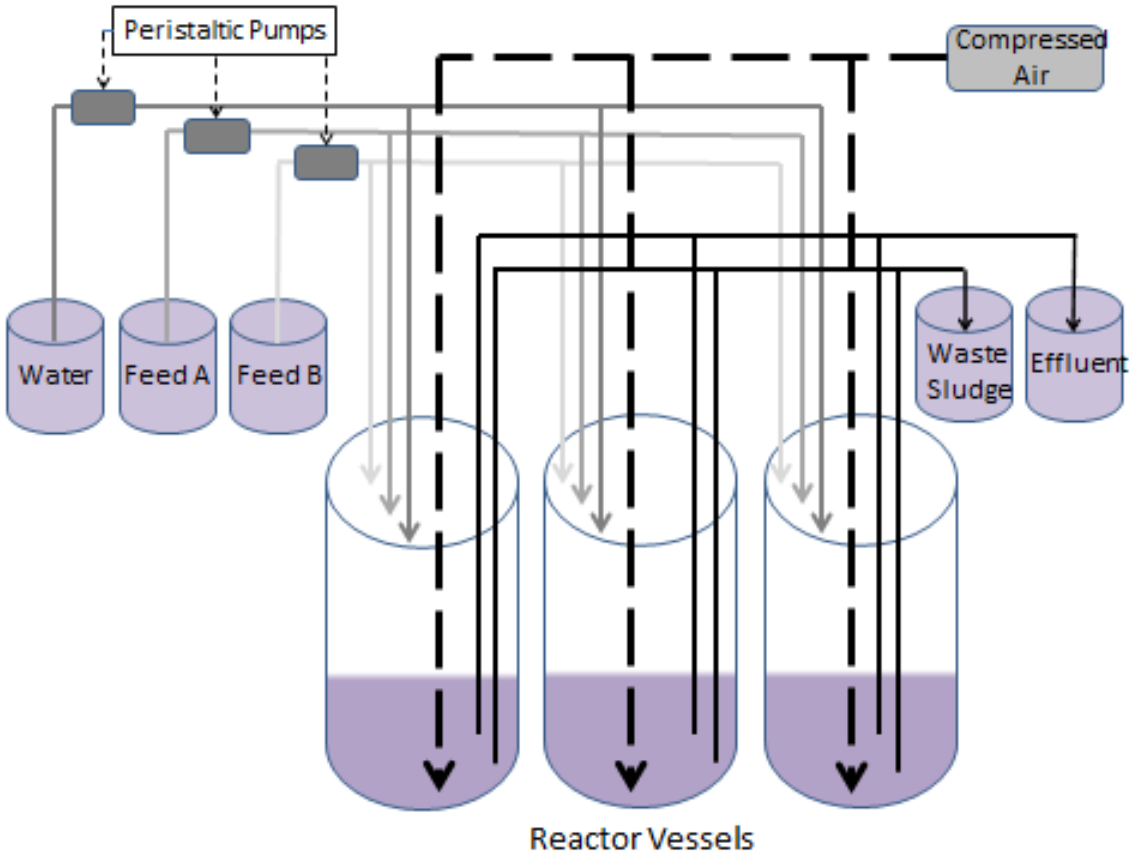

Figure 2: SBR setup

Solid and Liquid Phase Extraction

Malathion was extracted directly from the solid and liquid phases of the mixed liquor by filtering a 10 mL sample with 1.2 µm Whatman GF/C glass fiber filter paper. The filtrate was collected in a 50 mL amber-colored glass vial. Two mL of methylene chloride were added to the vials. The vials were parafilmed, vortexed, and allowed to sit for one hour in order to extract the malathion from the water to the methylene chloride.

The sample was then transferred with a Pasteur pipette to a 1.5 mL amber glass auto sampler vial. To extract malathion from the biomass solids, the Whatman GF/C filter paper holding the biomass solids was placed in a beaker. Four mL of methylene chloride was added to the beaker. The beakers were then covered with parafilm and placed in a Branson sonicator for 10 minutes. After sonication, the methylene chloride in the beaker was transferred via Pasteur pipette to a 1.5 mL amber glass auto sampler vial. Once the samples were placed in the 1.5 mL amber glass auto sampler vials, 20 μL of 100 ppm dimethyl methylphosphonate (DMMP) in methanol were added to the solid and liquid phase samples as an internal standard.

GC/MS

Sample analysis was conducted using an Agilent GCMS-6890N/5973 inert with a DB-35 mass spectrometer column (0.15μm film thickness, 0.25 mm id. x 30 m length, J&W Scientific, Folsom, CA, U.S.A.). Detection and quantification of malathion was accomplished in selected ion monitoring (SIM) mode to increase signal to noise while minimizing sample run time. A standard tune was performed on the mass spectrometer using bromofluorobenzene and the monitored ions for malathion and DMMP had *m/z* ratios of 125 and 124, respectively. The calibration curves, prepared with each experiment to ensure accurate quantification, were created using the ratio of malathion peak area (retention time 11.8 min) to the summed area of the DMMP isomer pairs. DMMP peaks appeared at 4.2 and 6.8 minutes due to DMMP dimerization, and the total run time was 12.5 minutes.

The initial column temperature was 50°C for two minutes and increased at a rate of 20°C/min to 260°C. The injection volume was 4 µl, and the syringe was rinsed with the sample six times before injecting the sample into the GC column. After sample injection, the syringe was rinsed three times with dichloromethane and three with methanol before the next sample began.

Sorption Kinetics

Sorption kinetic experiments were intended to determine the amount of time necessary for the AS to achieve maximum sorption of malathion. A 200 mL sample of AS was heat inactivated in an oven at 80° C for 30 minutes in order to inhibit biological degradation. At this temperature, the ribosomes of bacteria denature (Lee & Kaletunc, 2002) with minimal changes in AS features (Ren et al., 2007). Next, 10 mL of well-mixed AS was transferred to nine test tubes each. A tenth test tube was prepared with DI water to serve as the control. Each sample was injected with 10 µL of 1000 mg L^{-1} malathion solution. The samples were then placed on a tube rotator and removed at intervals ranging from two to 30 minutes. Once removed from the rotating disk, the samples were passed through a Büchner funnel with 1.2 µm Whatman GF/C glass fiber filter paper to separate the solid and liquid phases. The malathion was then extracted from the solid and liquid phases, as described above. The samples were compared to the control containing only water and malathion in order to account for the amount of malathion sorbed to the filter paper. A two-tailed statistical analysis of variance (α=0.05) was conducted to determine if AS malathion sorption differed statistically from filter paper malathion sorption. All measurements and tests were conducted in duplicate.

Sorption Equilibrium Isotherm

The goal of this experiment was to determine sorption equilibrium coefficients. Two hundred fifty mL of heat-inactivated (80°C for 30 min) AS was distributed to Erlenmeyer flasks in dilutions of 1530, 830, 760, 560, and 270 mg L^{-1}. The TSS/VSS concentrations were measured for each dilution of AS. A methanolic solution of malathion was added to each flask for final concentration of 1.66 mg L^{-1}. The flasks were placed on stir plates for 30 min to ensure maximum malathion sorption was achieved. The malathion was then extracted from the solid and liquid phases of the AS. Hydrolysis was minimized by maintaining the pH of the AS between 6 and 7. All measurements and tests were conducted in duplicate.

Biodegradation

The purpose of the degradation experiment was to determine the capacity of the AS to degrade malathion. This experiment was conducted with batch tests using three separate Erlenmeyer flasks, each filled with 800 mL of AS. Two of the flasks were exact duplicates containing AS, feed A, feed B, and malathion. The third flask served as the control flask containing only AS and feed, but no malathion. The flasks were aerated throughout the batch test. Feed A (3.2 mL) and feed B (15.2 mL), were added to the flasks at the beginning of the experiment in volumes proportionate to those fed to the SBR. Malathion (4.25 mg L^{-1}) was also added to two of the flasks at the beginning of the experiment. Samples were taken each hour from the flasks to measure concentrations of COD, ammonia (NH_3-N), nitrate (NO_3-N), nitrite (NO_2-N), and malathion from the solid

and liquid phases. COD, NH_3-N, NO_3-N, and NO_2-N were measured to monitor the performance of the AS heterotrophic and nitrifying bacteria. All measurements were conducted in duplicate.

Reactor pHs were maintained between 6 and 7 approximately. The pH in the reactors was measured at the beginning and end of each 12 hour cycle. The pH was manually adjusted twice daily with Feed A.

Biodegradation with Inhibition of Nitrification

The purpose of this experiment was to determine the role of nitrifying bacteria in the degradation of malathion by AS. This experiment was identical to the degradation experiment with one exception: 80 μM (10 mg L^{-1}) allylthiourea (ATU) was added to the AS to inhibit nitrification (Konig et al., 1998). In addition, a fourth flask containing only water and malathion was prepared as another control to account for abiotic effects such as volatilization, losses to glassware, and losses during the extraction process. ATU was initially added 12 hours prior to the beginning of the experiment to ensure adequate time for nitrification inhibition. An additional 10 mg L^{-1} ATU was added immediately prior to the test start time in order to ensure inhibition of nitrification for the duration of the experiment. ATU is believed to bind with the copper of the AMO active site, and therefore selectively inhibits nitrification (Bédard & Knowles, 1989). While ATU can inhibit nitrifiers at concentrations as low as 8 μM (Hofman & Lees, 1953; Tomlinson et al., 1966; Hooper & Terry 1973; Sharma & Ahlert, 1977), complete inhibition can be achieved at an ATU concentration of 86 μM without affecting other metabolic activities (Ginestet et al., 1998) with the exception of other monooxygenases. Reactors pHs were

maintained between 6 and 7. A two-tailed statistical analysis (α=0.05) was conducted to determine if malathion degradation with all bacteria active was statistically different from the malathion degradation with the nitrifiers inactive. Measurements were conducted in duplicate.

Other Analytical Methods

Hach methods 8000, 10031, 10020, and 8153 were utilized to measure COD, NH_3-N, NO_3^--N, and NO_2^--N concentrations. TSS and VSS were measured using standard methods (APHA, AWWA, WEF, 1998). All measurements and tests were conducted in duplicate.

Results and Discussion

Sorption Kinetics and Equilibrium Isotherms

The mean AS malathion sorption did not differ statistically compared to the mean filter paper malathion sorption (p=0.052). AS malathion sorption remained constant with contact time (Fig 3) and varying TSS concentration (Fig 4). Therefore, sorption would not be a viable removal mechanism for malathion in a municipal WWTP. Further, data were analyzed using the Freundlich linear and Langmuir adsorption isotherm equations (Figs 13 and 14), which suggest that adsorption is insignificant under the experimental conditions investigated. These results agree with the observation that the log K_{oc} value of malathion has been reported as 3.07 (Aleksandar et al., 1995), which predicts that malathion will be less likely to sorb onto biomass and therefore more likely to remain in the liquid phase. Similarly, the log K_{oc} for VX is 2.52 (Davisson et al., 2005). The

biomass is not acting like something that adsorbs malathion. The larger the K_{oc}, the more likely adsorption will occur, therefore; VX would be expected to adsorb even less. Malathion recovery ranged between 70% and 90%.

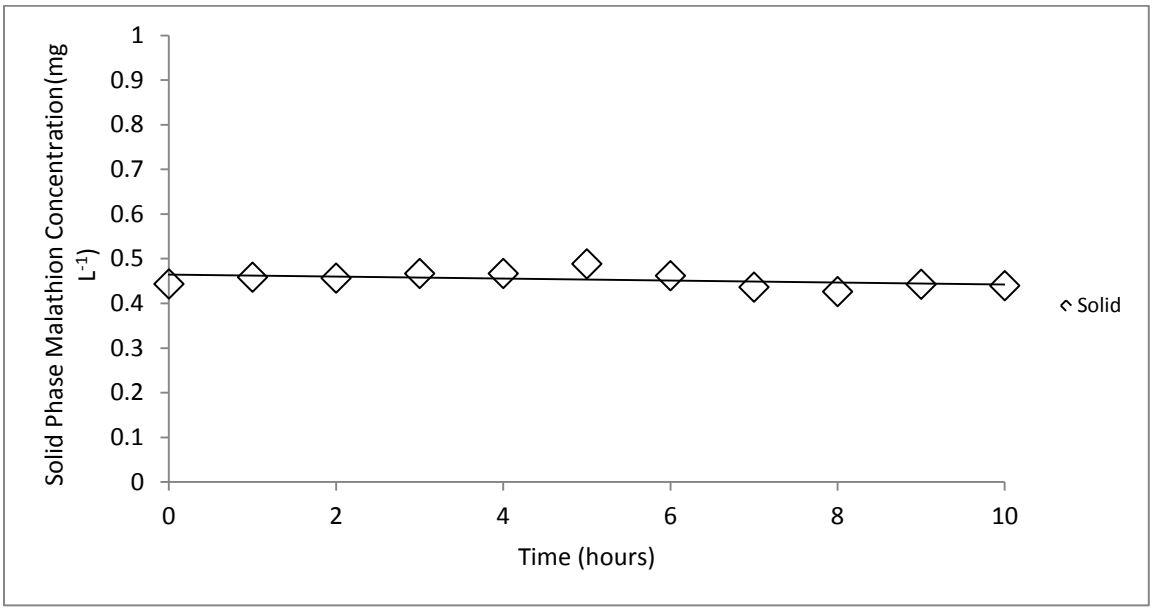

Figure 3: Malathion AS Sorption: 4.25 mg L^{-1} inactivated sludge at 25° C

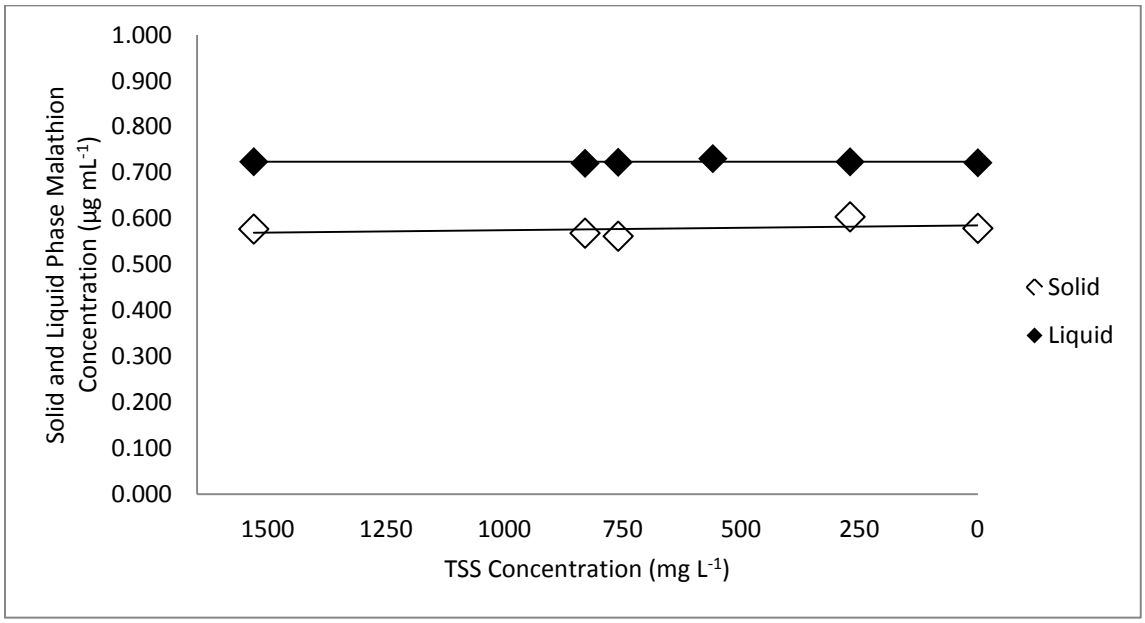

Figure 4: Malathion Sorption Isotherm: 1.66 mg L^{-1} inactivated sludge at 25° C

It was assumed that the losses due to volatilization and photolysis were negligible because malathion has a low vapor pressure and Henry's constant (Table 1), and photolysis was limited with the use of amber glassware or storing the chemicals in limited light. OP compounds are known to undergo abiotic transformations in water caused by base catalyzed hydrolysis (Wolfe et al., 1990). Malathion hydrolysis is pH and temperature dependent (Bartelt-Hunt et al., 2008; Brown et al., 1993; Newhart, 2006). Hydrolysis is the main route of degradation in alkaline aerobic conditions and may be a significant environmental degradation route (Bartelt-Hunt et al., 2008; Brown et al., 1993; Newhart, 2006). Malathion hydrolysis is slow compared to biodegradation when the pH<7 and malathion is slowest at pH 4 (Bartelt-Hunt et al., 2008; Brown et al., 1993; Newhart, 2006). Under acidic conditions, potential hydrolysates formed are malathion monocarboxylic and dicarboxylic acid, diethyl thiosuccinate, diethyl thiomalate, and O, O-dimethyl phosphorothionic acid (Bartelt-Hunt et al., 2008; Newhart, 2006). Under alkaline conditions, malaxon, malathion monoacid, diethyl fumarate, diethyl thiomalate, ethyl hydrogen fumarate, O, O-dimethylphosphorothionic and O, O-dimethyl phosphorodithioic acid may be formed (Bartelt-Hunt et al., 2008; Brown et al., 1993; Newhart, 2006).

Biodegradation

The liquid phase malathion concentration decreased by approximately 90% in each of the biodegradation experiments. The malathion concentration continuously decreased over the 12 hour experiment from a concentration of approximately 4.25 mg L^{-1} to approximately 400 μg L^{-1} (Figs 5 and 6). Simultaneously, the NH_3-N concentration

decreased from 14 mg L^{-1} to 0 mg L^{-1} and the NO_3-N concentration increased from 40 mg L^{-1} to 140 mg L^{-1} within 12 hours, indicating nitrification was occurring. The COD concentration, however, did not decrease as it had prior to the addition of malathion. Whereas before the addition, the COD effluent concentration was approximately 10 mg L^{-1}, the effluent COD concentration fluctuated between 30 mg L^{-1} and 170 mg L^{-1} after malathion was added. Therefore, the malathion did not inhibit nitrification, though may have affected COD oxidation.

There are four potential causes for the increased COD concentrations near the end of the biodegradation experiments. First, it is possible that the malathion stressed the AS, leading to cell lysis and passive release of intracellular soluble microbial products (SMP) (Henriques & Love, 2007; Barker & Stuckey, 1999). However, nitrification was not affected in the biodegradation experiment with all bacteria active, and autotrophic nitrifiers are sensitive to toxic shock (König et al., 1998). Second, the fluctuating COD concentrations could be the result of active excretion of SMP as a response of a toxic exposure. SMP may be excreted in response to a toxic substance or to establish concentration equilibrium across the cell membrane (Barker & Stuckey, 1999). Third, the COD is a release of materials from the extracellular polymeric substances (EPS) matrix in which cells in AS flocs are embedded (Henriques & Love, 2007). Fourth, the inconsistent COD oxidation may be a stress response. AS may respond to stress by diverting energy from growth to managing the stress condition (Villain & Marrot, 2013).

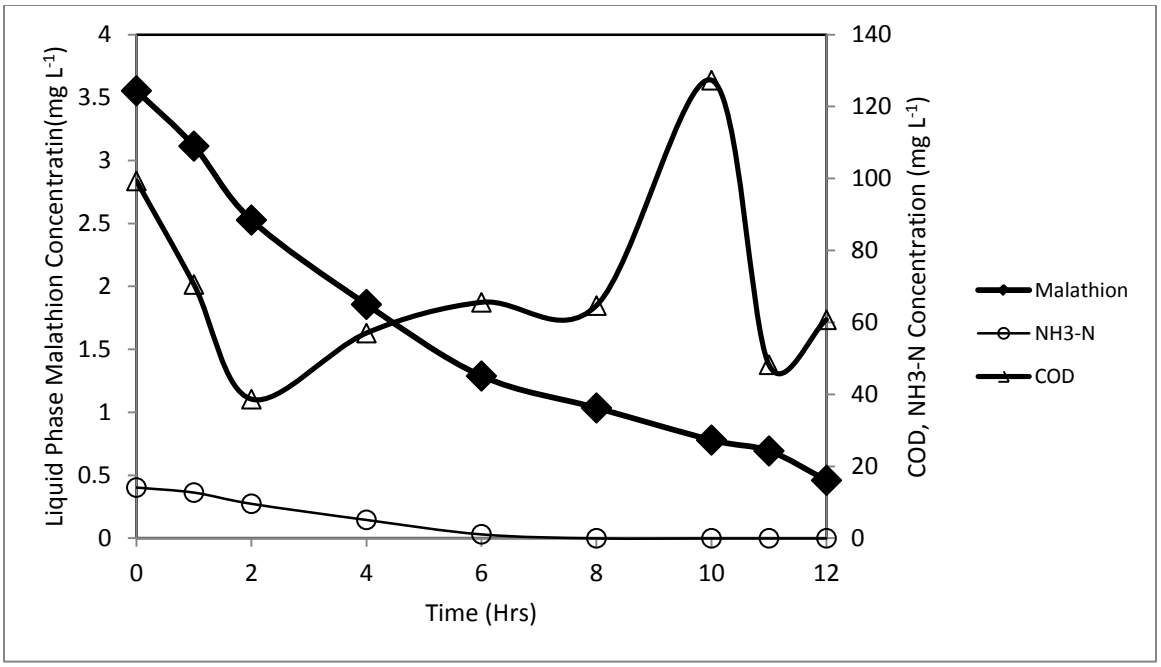

Figure 5: Malathion Biodegradation: 4.25 mg L^{-1} influent AS concentration at 25° C with nitrifiers active

In the second biodegradation experiment, 80 μM of ATU was added to each flask containing AS to determine the role of the nitrifying bacteria in the degradation of malathion (Fig 6). A fourth flask containing only DI water and malathion was added to account for hydrolysis and other abiotic effects. The biodegradation of malathion was unaffected by the inhibition of the nitrifying bacteria as the malathion concentration again decreased by 90 percent. The mean malathion degradation with all bacteria active did not statistically differ from mean malathion degradation concentrations with the nitrifying bacteria inactive ($p=0.546$). Since the inhibition of the nitrifying bacteria did not affect liquid phase malathion degradation, the heterotrophic bacteria were likely largely responsible for degradation.

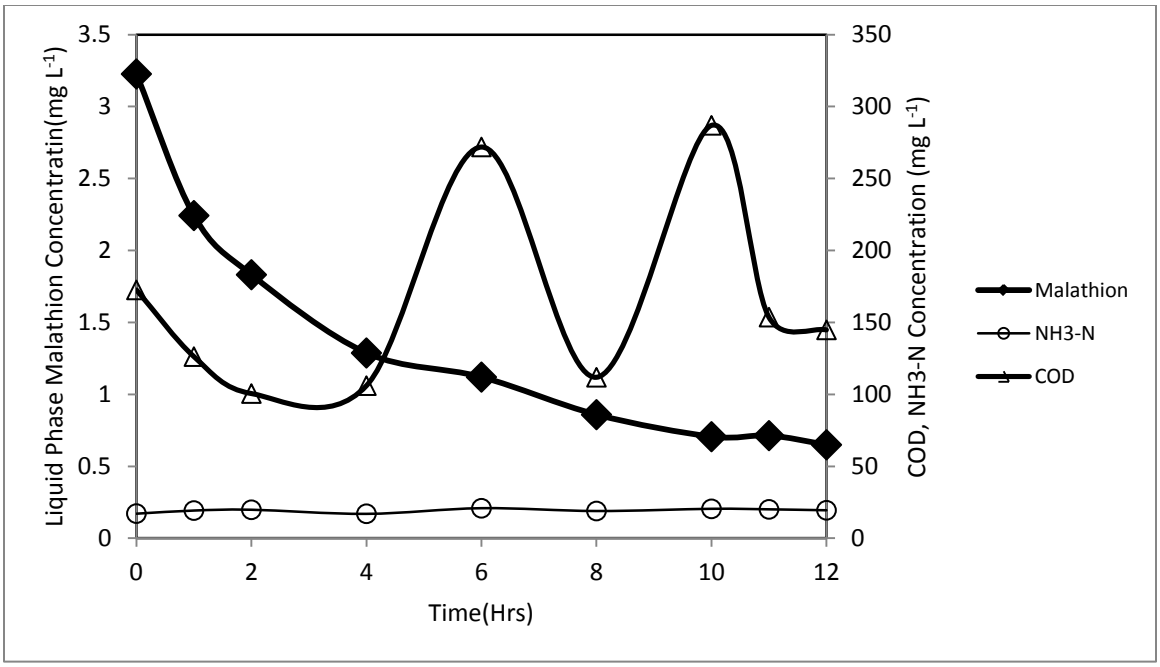

Figure 6: Malathion Biodegradation: 4.25 mg L^{-1} AS at 25° C with nitrifiers inhibited

Some degradation may be attributed to abiotic affects such as photolysis and hydrolysis (Fig 7). A two-tailed statistical analysis (α=0.05) was conducted to determine if abiotic malathion degradation statistically differed from biotic malathion degradation. The mean abiotic malathion degradation differed statistically from the mean biotic malathion degradation with the nitrifying bacteria inactive (p=0.000). Abiotic effects accounted for 40% of the malathion degradation. As stated earlier, the pH was maintained between 6 and 7 approximately. Hydrolysis is the main route of malathion degradation in water with pH > 7.0 (Wolf et al., 1990; Newhart, 2006). When pH <7.0, the rate of hydrolysis is slow relative to the rate of biodegradation (Newhart, 2006). Love reported similar VX hydrolysis results: slower hydrolysis at low pH conditions and

greater hydrolysis at high pH conditions (Love et al., 2004). Hydrolysis and photolysis may be potential removal mechanisms.

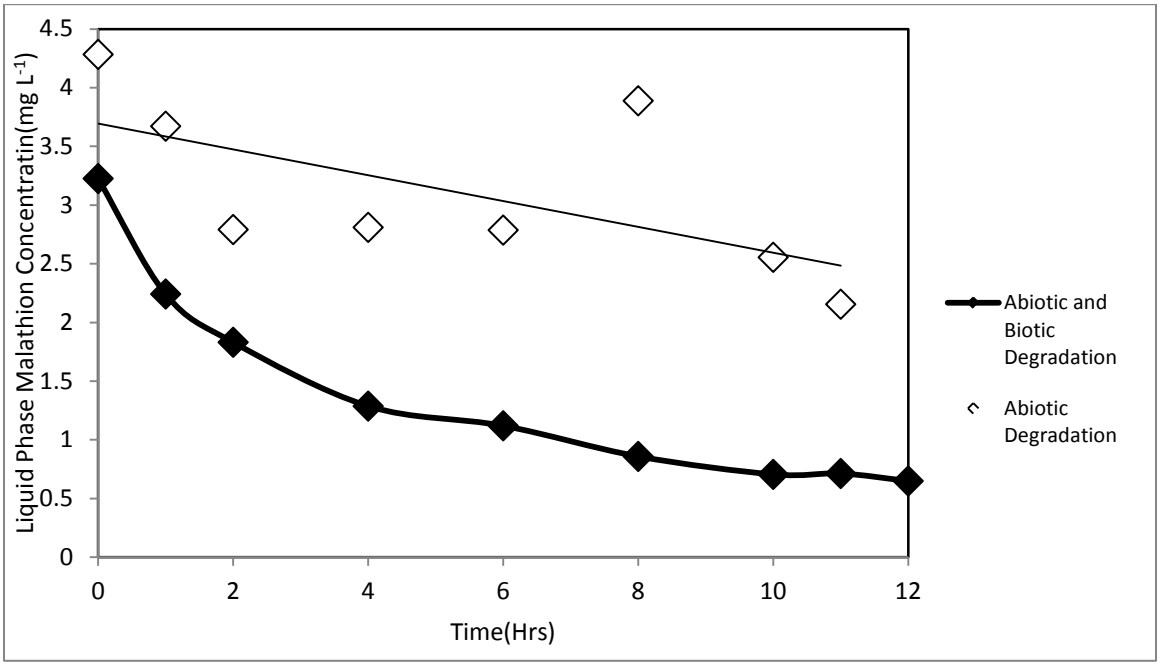

Figure 7: Malathion Abiotic and Biotic Degradation: 4.25 mg L^{-1} AS at 25° C

Four compounds were routinely detected in our experimental samples containing malathion. The compounds were tentatively identified as isomalathion (ISO), O,O,S-trimethyl phosphorodithioate (OOS(S)), O,O,O-trimethyl phosphorothioate (OOO(S)), and diethyl fumarate. The manufacture of malathion results in the production of these impurities (Brown et al., 1993; Ambrus et al., 2003); however, diethyl fumarate is also considered a hydrolysis product (Bartelt-Hunt et al., 2008; Brown et al., 1993; Newhart, 2006). In this instance, the diethyl fumarate was likely a product of hydrolysis as the compound was not observed in samples taken at time = 0 hours immediately after the addition of the malathion. According to the manufacturer, technical grade malathion concentrate contains 0.2% isomalathion, 0.9% diethyl fumarate, 1.2% OOS(S), and

0.45% OOO(S) (Brown et al., 1993). Isomalathion, OOS(S), and OOO(S) are significantly more potent inhibitors of mammalian acetylcholinesterase than malathion, with relative LD_{50}'s measuring 95, 178, and 17 times greater in rats (Brown et al., 1993; Aldridge et al., 1979). Figure 8 suggests these compounds were degraded in the AS as well.

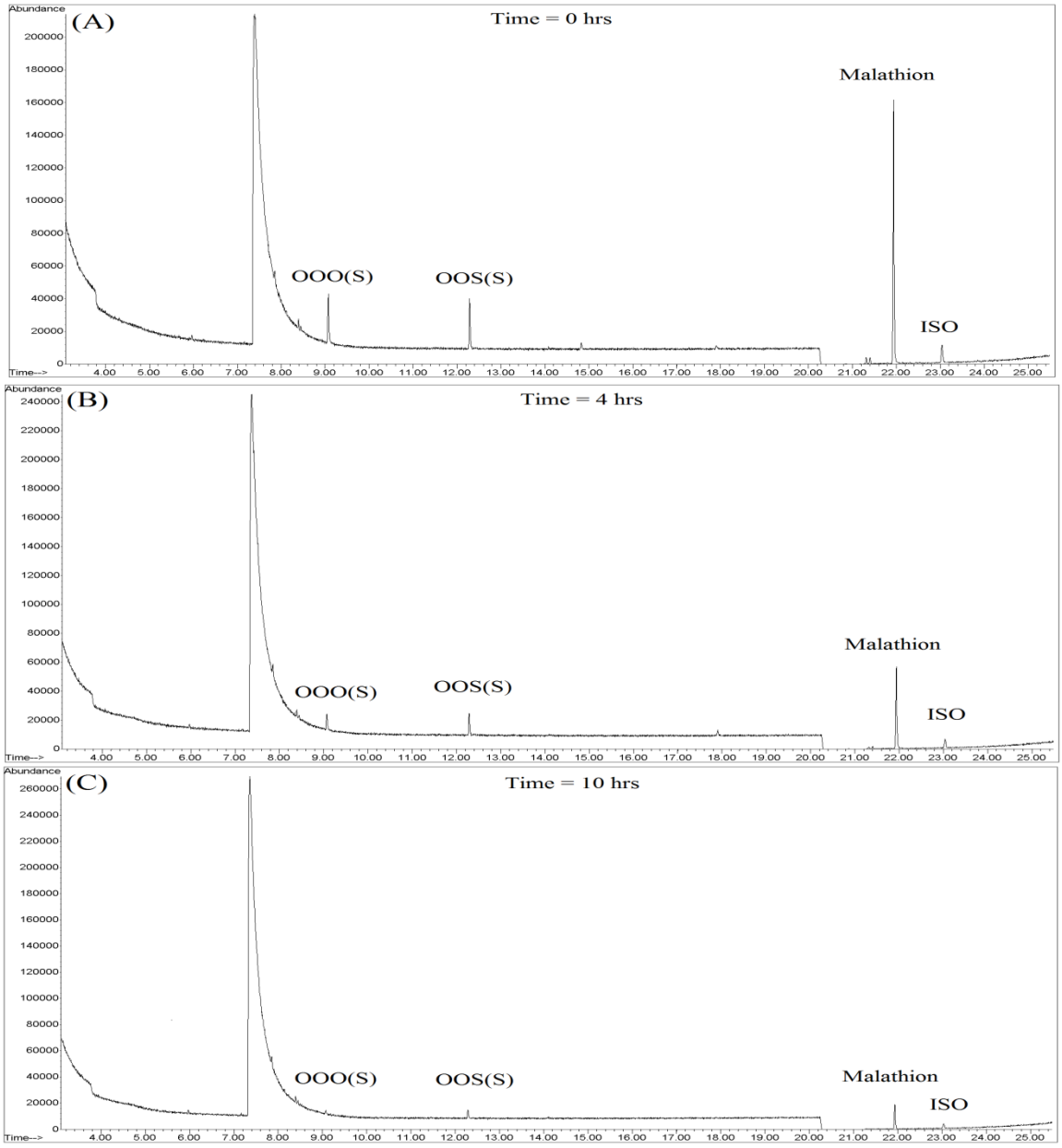

Figure 8: GCMS Chromatograph of Impurity Degradation at (A) 0 hrs, (B) 4 hrs and (C) 10 hrs

Conclusions

These results suggest that if malathion were to enter a municipal WWTP, much of it may be degraded mitigating the risk of release in the effluent. Nitrification would not likely be affected by the toxic load. However, COD removal may be inhibited or become

unstable which presents new risks. Furthermore, sludge wasting would not be an effective removal mechanism as sorption to the AS is negligible.

This study demonstrates that malathion may largely degrade when exposed to a typical municipal wastewater treatment process, both biotically and abiotically. Sorption kinetics and isotherm experiments resulted in negligible malathion sorption to AS minimizing the potential for sludge wasting as a removal mechanism, and the contamination of sludge by malathion. Approximately 90% of the initial 4.25 mg L^{-1} influent malathion concentration was biodegraded, mostly likely by heterotrophic bacteria. Abiotic effects accounted for 40% of the malathion degradation, making hydrolysis and photolysis potential removal mechanisms. Nitrification was not affected by the toxic load. However, COD oxidation was negatively impacted.

III. Conclusions

Chapter Overview

This section summarizes the results and corresponding findings that answer the research questions. The significance of this research will be highlighted as the findings are reviewed and areas for future research are identified.

Review of Findings

The research results demonstrate that malathion will degrade when exposed to a typical municipal wastewater treatment process. Approximately 90% of the initial 4.25 mg L^{-1} influent malathion concentration was biodegraded. Nitrification was not affected,

though COD oxidation may have been inhibited. Abiotic effects accounted for 40% of the malathion degradation. Malathion sorption to AS was negligible.

Significance of Research

If a CWA incident occurred, contaminated personnel and property would likely be decontaminated with a water-based solution. The contaminated wastewater could largely be contained. However, it is conceivable that the wastewater may ultimately be sent to a municipal WWTP for treatment. This research demonstrates that the majority of malathion will be degraded in the aeration phase of a typical wastewater treatment process. Assuming VX and its hydrolysis products degrade similarly to malathion, there may be mitigated risk of downstream contamination of those specific OPs.

Limitations

VX and malathion share many physical and chemical characteristics. However, these OPs may not behave exactly the same in a municipal WWTP. Therefore, the conclusions from this research may not apply directly to VX. Conducting research with VX was beyond the capability of the AFIT Laboratory and this research project. Similarly, lab studies are intended to mimic natural field conditions on a microcosmic scale and provide the researcher a means to easily adjust those conditions in order to achieve a desired goal. Field conditions are considered uncontrolled when compared to lab conditions which limits direct application of specific data presented above. Namely, characteristics such as influent characteristics, sludge retention time, and toxic load may change often in a municipal treatment plant which affects the composition of the bacterial

community. Furthermore, the synthetic feed was a wastewater stimulant, potentially altering the makeup of the bacterial community from a full-scale plant.

Nevertheless, the results provide important insights to the probable behavior of OP compounds in AS. In the event of a CWA, personnel and equipment will likely be decontaminated with water. Any decontamination wastewater or CWA runoff entering a municipal treatment plant would be extremely dilute. VX, like malathion, has many pH dependent hydrolysis products. The hydrolysis compounds may be equally or more hazardous. There is limited documentation on the fate of these hydrolysis products in a WWTP. No abiotic degradation products of malathion were identified during this research, as it was not the focus of this research project.

Future Research

A longevity experiment should be conducted to determine the long-term effects of malathion on the AS. An experiment that feeds a constant concentration of malathion over the course of an extended period of time would demonstrate whether the long-term health of the community would be negatively impacted. Continuous introduction of the toxic load could affect degradation ability, bacterial community composition, bacteria growth, or cause cell death. Once accomplished, a higher concentration should be evaluated to ensure similar performance. Process characteristics such as sludge retention time should be varied as well to identify the TSS which yields maximum degradation. In most cases, a longer SRT corresponds to a higher AS concentration and a lower effluent concentration of biodegradable substrates (Rittmann & McCarty, 2001).

The research results suggest abiotic effects, hydrolysis and photolysis, may be a potential removal mechanism for malathion and possibly VX. An experiment measuring malathion degradation at various wavelengths of light may provide more insight to the potential to utilize photolysis as a removal method. UV light is currently used in some WWTP's as a tertiary treatment for bacterial disinfection. In addition, hydrolysis is temperature and pH dependent. There is potential for increased hydrolysis rate by increasing the pH and temperature of the system.

Sorption kinetics and degradation experiments should be conducted on VX itself. While surrogates provide a safer alternative for research on toxic materials and can help establish boundaries to the experimental results, VX itself might provide the most accurate answers to these research questions.

Summary

This research explored the fate of malathion, a surrogate for the OP CWA, VX, in a municipal WWTP activated sludge system. The purpose of this research was to determine the ability of an activated sludge system to degrade malathion and avoid hazardous OP concentrations in the effluent. The research included a series of batch tests using activated sludge grown in three identical sequencing batch reactors, seeded with sludge from the FWRF. The data showed that malathion sorption was negligible. However, approximately 90% of the malathion degraded, biotically and abiotically, in the AS within 12 hours. Furthermore, it was determined that the heterotrophic bacteria were likely responsible for the degradation and the nitrifiers played a negligible role in the malathion degradation. Therefore, a conventional municipal AS wastewater treatment

process may be able to effectively degrade an OP CWA with characteristics to similar to malathion, though COD oxidation may be negatively impacted and degradation byproducts remain.

Appendix A. GCMS Calibration Curves

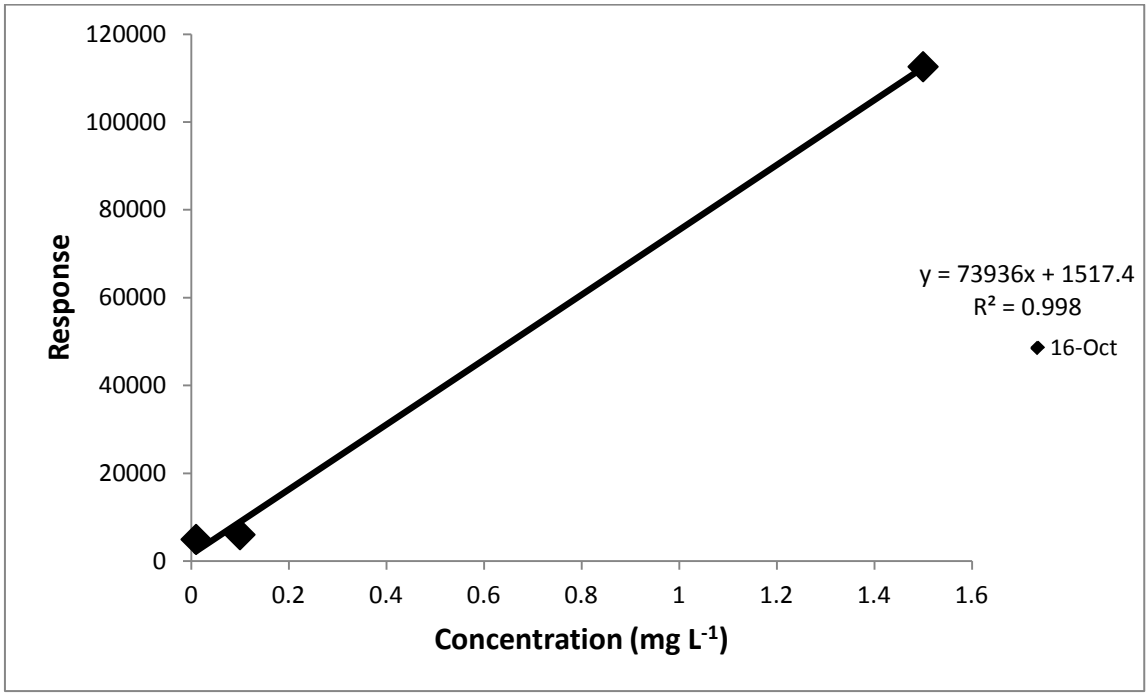

Figure 9: Calibration curve for sorption kinetics

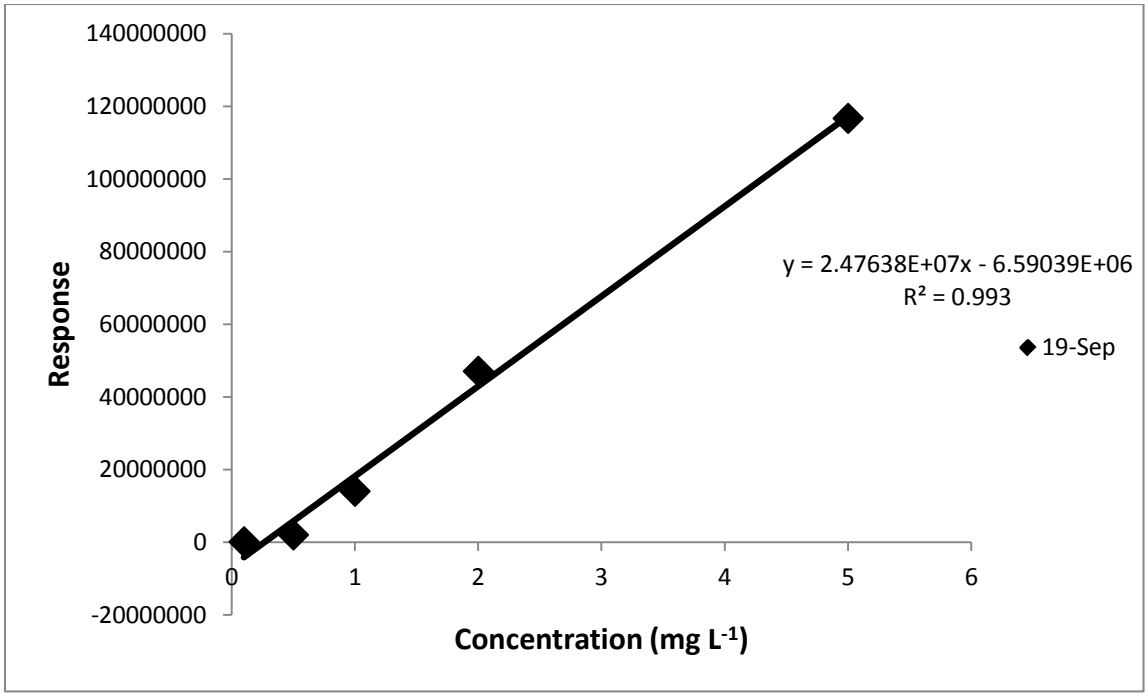

Figure 10: Calibration curve for sorption equilibrium isotherm

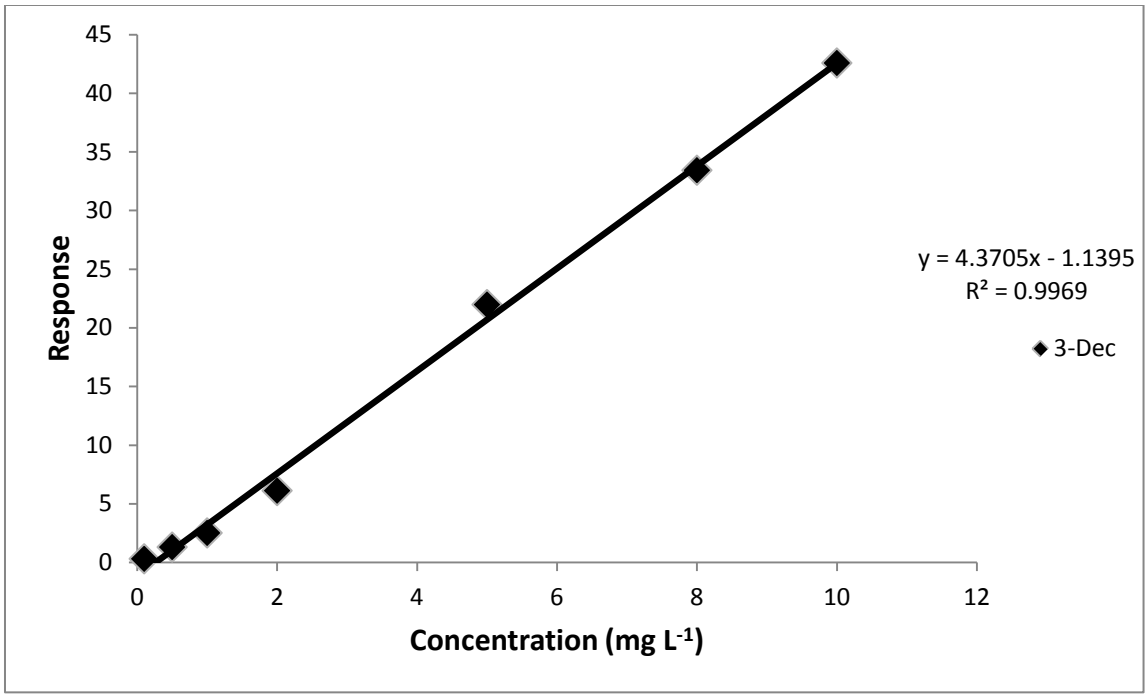

Figure 11: Calibration curve for degradation with nitrifiers active

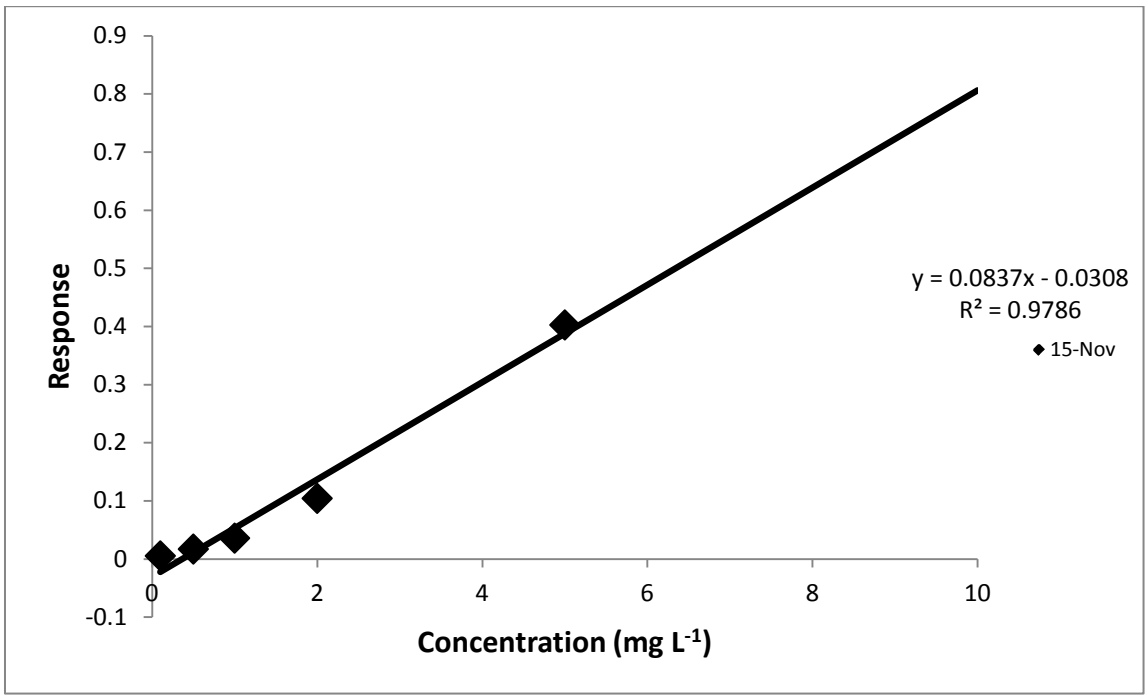

Figure 12: Calibration curve for degradation with nitrifiers inhibited

Appendix B. Sorption Equilibrium Isotherm

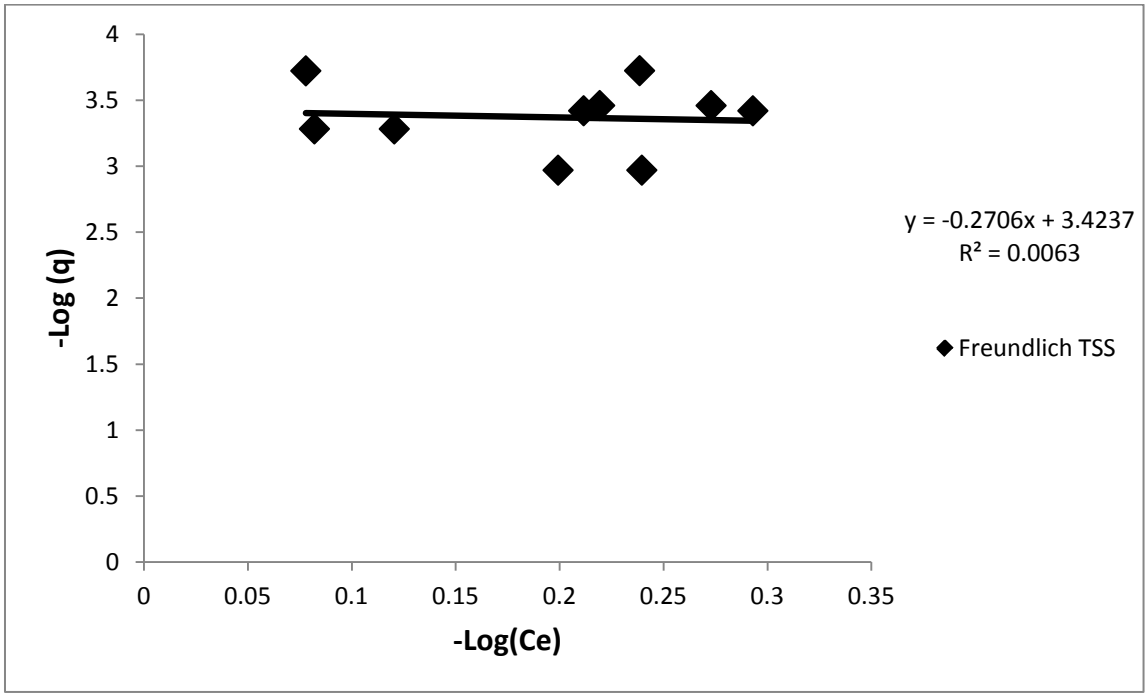

Figure 13: Sorption Equilibrium Isotherm: Freundlich TSS

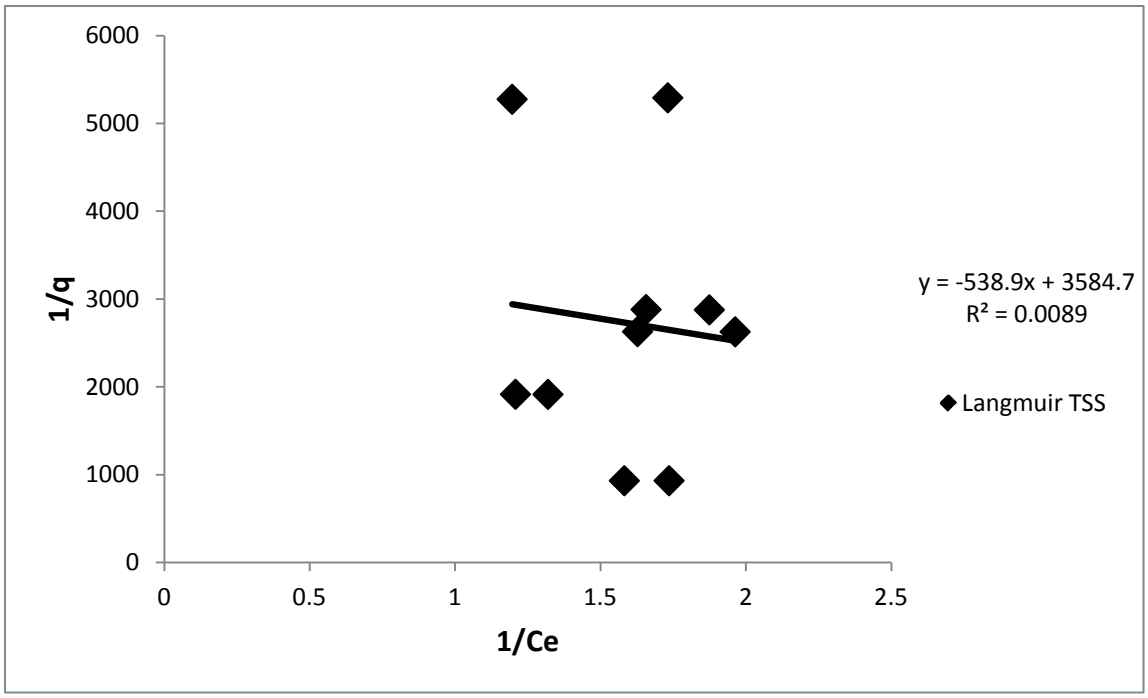

Figure 14: Sorption Equilibrium Isotherm: Langmuir TSS

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14. ABSTRACT

Organophosphate compounds are used as pesticides and in chemical warfare agents such as nerve agents. VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate) is one of the most toxic and environmentally persistent of these nerve agents. This research examined the fate of malathion, a pesticide and surrogate of VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate), in bench-scale activated sludge (AS) sequencing batch reactors. Sorption kinetics and sorption equilibrium isotherm experiments indicate that sorption to AS biomass was not a statistically important removal mechanism. However, approximately 90% of the initial 4.25 mg L^{-1} malathion concentration degraded primarily via heterotrophic activity.

15. SUBJECT TERMS
Organophosphate chemical warfare agents, malathion, activated sludge, VX biodegradation

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